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“Preliminary Note on the Use of Chloroform in the Preparation of Vaccine.” By ALAN B. GREEN, M.A., M.D. (Cantab). Communicated by W. H. POWER, M.D., F.R.S. Received April 16,—Read April 30, 1903.

(From the Government Lymph Laboratories.)

It is well known that glycerine exerts an action on vaccine whereby the extraneous bacteria are eliminated in the course of a few weeks, while the specific germ undergoes no undue deterioration from the process.

I have found that by the use of a solution of chloroform in distilled water, the extraneous bacteria of vaccine are eliminated in from one to six hours, the specific germ remaining fully potent for vaccination.

The solution of chloroform that can most advantageously be employed in the preparation of vaccine is a saturated solution in distilled water, having a strength of 1 in 200. This is the limit of such solubility.

The following method of using such solution has so far given the best results :—

Vaccine emulsion is first prepared by triturating vaccine pulp with distilled water. *The presence of the water is essential*, in order that later chloroform may enter into solution with it. About three parts by weight of water should be mixed with one part by weight of pulp. Should a more viscid emulsion of vaccine be desired, glycerine may be added without interfering with the action of the chloroform. I have found that the usual admixture of one part by weight of vaccine pulp and four parts by weight of a solution consisting of equal parts by weight of glycerine and water forms a perfectly suitable emulsion for this process. But glycerine is incapable of dissolving chloroform, and the elimination of extraneous bacteria by this chloroform process

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is solely due to the action of chloroform water. Indeed, when addition of glycerine to the vaccine emulsion is desired, it can be very advantageously effected after the completion of this process.

The newly-made vaccine emulsion, to be subjected to the action of chloroform, is dealt with in the following way:—Sterile air is first passed through pure liquid chloroform, whereby this air becomes charged with chloroform vapour. This mixture of air and chloroform vapour is then passed through the vaccine emulsion, which is contained in a cylindrical glass vessel of test-tube shape, and in size suitable to the quantity of vaccine to be treated.

The mixed chloroform vapour and air can be passed seriatim through a number of tubes of vaccine before it finally escapes into the outside air, and it is efficient for all of them, provided that the current be sufficiently strong to keep the contents of each tube in active movement, and that a distinct smell of chloroform be apparent at the outlet of the last tube of the series.

It is essential that no liquid chloroform be allowed to pass over into the vaccine, as its presence is strongly inimical to the potency of the lymph. To obviate the chance of such an accident an overflow bottle, weighted with sterile sand, is interposed between the bottle of liquid chloroform and the tube or tubes of vaccine emulsion.

By passage through it of chloroform vapour and air, the water of the vaccine emulsion quickly becomes saturated with chloroform, and this strength of solution is maintained so long as such passage is continued. When saturation is reached all excess of chloroform immediately escapes automatically from the vaccine. Thus the vaccine is not at any time brought into contact with a stronger solution of chloroform than 1 in 200 in water.

A rapid and marked germicidal action is exerted on the non-spore-bearing extraneous bacteria of vaccine thus treated. The extraneous bacteria most commonly found in vaccines at the Government laboratories are *Staphylococcus pyogenes aureus*, *Staphylococcus pyogenes albus*, *Staphylococcus cereus flavus*, *Staphylococcus cereus albus*. Others which occur either in smaller numbers or less commonly are *Staphylococcus pyogenes citreus*, *Proteus vulgaris*, *Streptococcus pyogenes*, *Sarcina lutea*, and some yeasts. Emulsions, which have contained as many as 100,000 extraneous micro-organisms per platinum loopful at the time of mixture, have, by the action of chloroform water, become free from their presence in from 1—6 hours. This freedom is evidenced by absence of bacterial growth in aërobic and anaërobic plate cultures. The germicidal action is first exerted on the least resistant members of each species of organism present in the vaccine. Generally after the first hour or hour and a half of the process, a very few of the more resistant staphylococci—*aureus* and *albus*—remain alive; these give rise to small inhibited colonies in plate cultures. And these

organisms succumb in their turn after further application of the process.

By contrast, elimination in like degree of the extraneous micro-organisms of vaccine by the glycerine process rarely occurs before the fourth week after mixture, and is frequently not complete until a much later period, as shown by similar plate cultures.

After elimination of extraneous bacteria from chloroformed vaccines, the chloroform is evaporated until no trace remains. Such evaporation is most quickly effected by passing a stream of sterile air through the emulsion.

By the above method vaccine can be brought under the influence of the germicide for such time only as suffices to kill the extraneous micro-organisms. At present, however, there is no evidence to show that more prolonged contact with 1 in 200 watery chloroform solution has any harmful effect on its potency.

As in the case of glycerine, non-spore-bearing bacteria in vaccine lymph are alone killed by this process. But in some thousands of vaccines examined at the Government Lymph Laboratories, the only spore-bearing organisms found in vaccine were the strictly non-pathogenic organisms of the mesenteric group—*Bacillus mesentericus vulgaris*, *Bacillus mesentericus fuscus*, *Bacillus mesentericus ruber*, *Bacillus subtilis*—and equally non-pathogenic moulds such as *Penicillium glaucum*.

The practical working value of the foregoing method has been clearly shown by results of vaccinations performed with vaccines which have been thus subjected to the action of chloroform. These vaccines, having been rendered free from extraneous micro-organisms, were first tested on calves and found to give excellent results. Within a fortnight after collection from the calf and of subjection to the action of chloroform water, such vaccines have been used (after evaporation from them of all chloroform) for primary vaccinations and re-vaccinations with results of high "case" and "insertion" success.

It would seem, therefore, that the following considerable advantages are to be gained by the use of the chloroform process :—

(a) So speedy an elimination of extraneous micro-organisms is attained that vaccine, practically free from such organisms, can be distributed for use within a few hours of its collection from the calf. In times of urgent demand for large quantities of vaccine, such as occur during small-pox epidemics, this process must needs prove of great value, since the necessity for wasting some weeks for elimination of extraneous organisms by glycerine will be done away with.

(b) In so far as the vaccination value of vaccine depends on the activity of a living organism, deterioration of that value must occur in the course of a longer or shorter time. The potency of some vaccines, glycerinated or otherwise, becomes greatly impaired within

a few weeks of collection, that is within the time required for glycerine to exert fully its influence in eliminating extraneous organisms. Some of these vaccines may, at the time of their collection, have possessed a high vaccination value. Vaccine, characterised by this high but somewhat transient potency, can, by means of the chloroform process, be used at once, before its activity has deteriorated, thus allowing greater economy of vaccine material than would otherwise be possible.

(c) For a similar reason the chloroform process might be of considerable use in hot climates where the preservation of the potency of vaccine is frequently a matter of considerable difficulty.

Experiments are at present being made to test the duration of the potency of chloroformed vaccines. A further account of this process will be given in the Report for 1902—1903 of the Medical Officer of the Local Government Board.

In conclusion I wish to express my indebtedness and thanks to Dr. F. R. Blaxall for the generous help and advice he has given me. My thanks are likewise due to Mr. H. S. Fremlin, with whom I am also associated in the work of these laboratories, and to Mr. S. D. Rowland, of the Jenner Institute of Preventive Medicine, for the help he has afforded me.

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